Fig. 1A

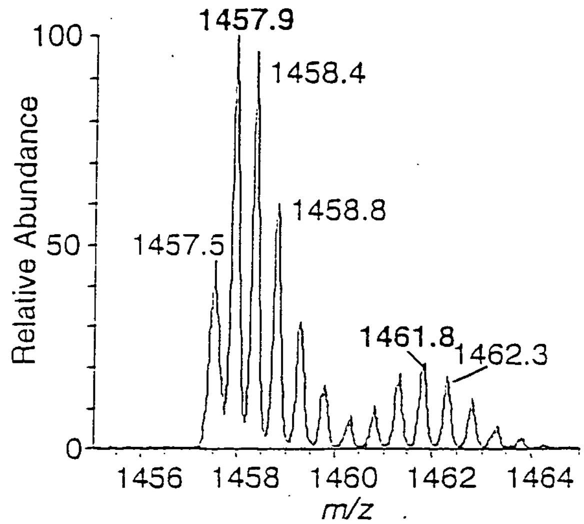
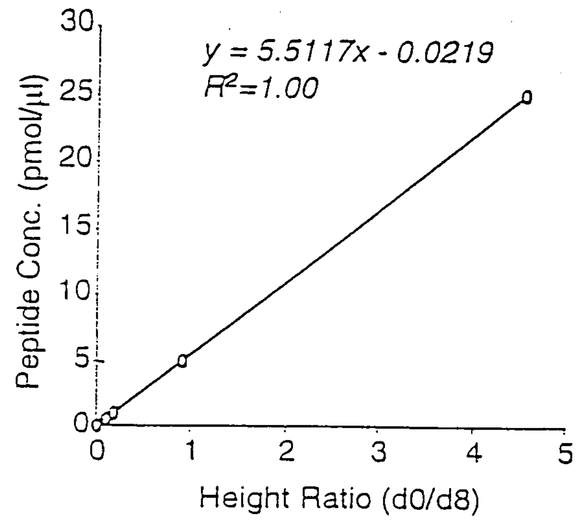
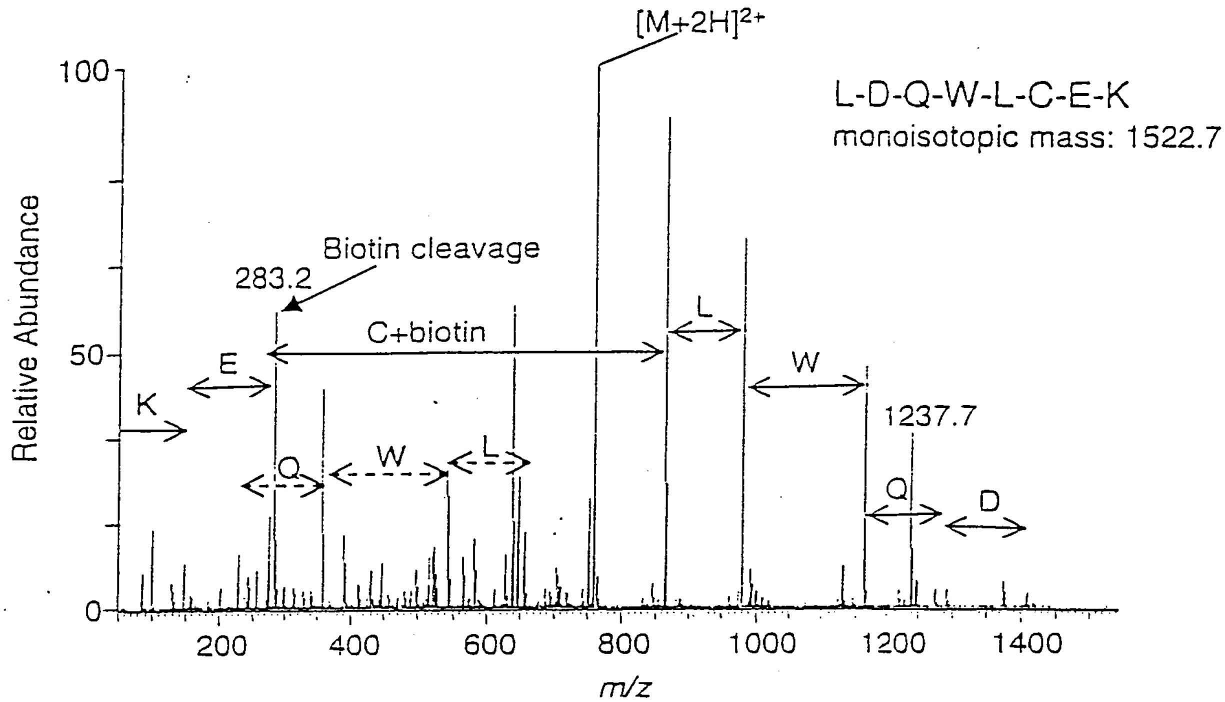


Fig. 1B



Standard curve generated with a cysteine-biotinylated peptide and quantitation by stable isotope dilution. **A)** Zoom-scan from an ion-trap mass spectrometer showing a 4 amu isotope distribution for the $[M+2H]^{2+}$ ions of the peptide modified with the isotopically light (1457.9 u) and heavy (1461.8) biotinylating reagents. The ratio (d0/d8) was 4.54. **B)** Curve generated from the analysis of isotope ratios from zoom-scans of 5 different concentrations of d0-labeled peptide measured in the presence of a known amount of peptide labeled with the isotopically heavy reagent.



Tandem mass spectrum of a cysteine-modified peptide from α-lactalbumin. Modification of the cysteine residue with the custom synthesized biotinylating reagent did not affect the ability of the Sequest computer program to correctly match this peptide to a database sequence.

Fig. 2

Fig. 3A

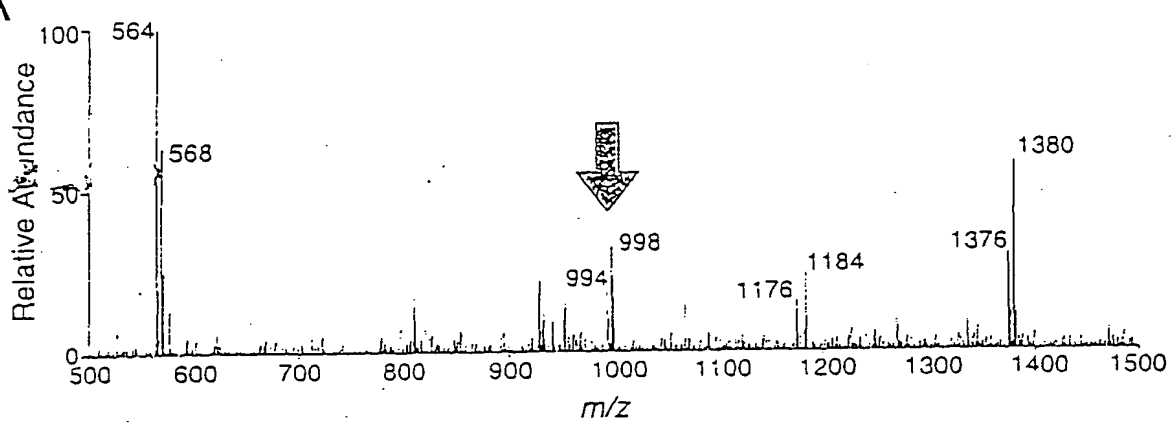


Fig. 3B

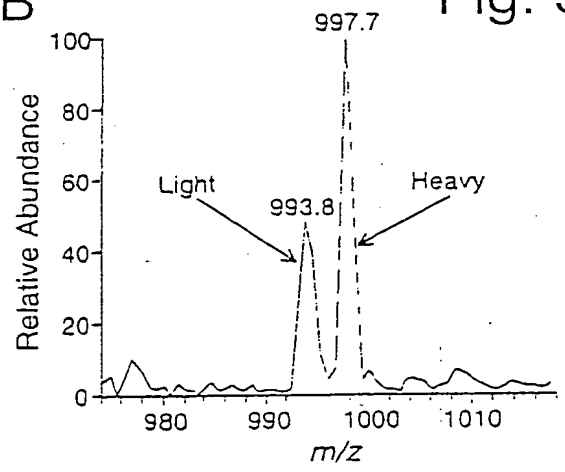


Fig. 3C

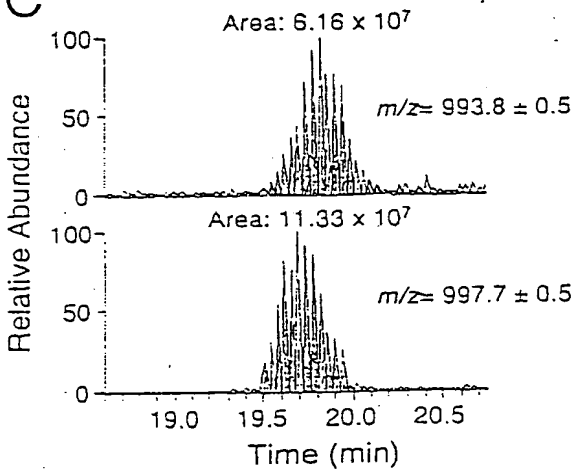


Fig. 4A

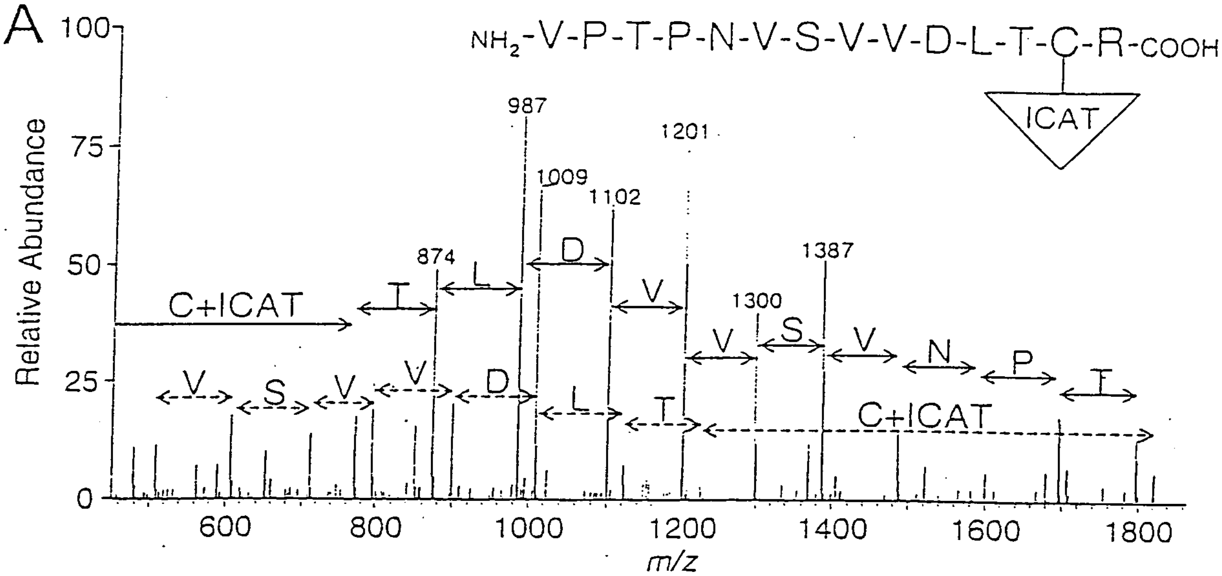
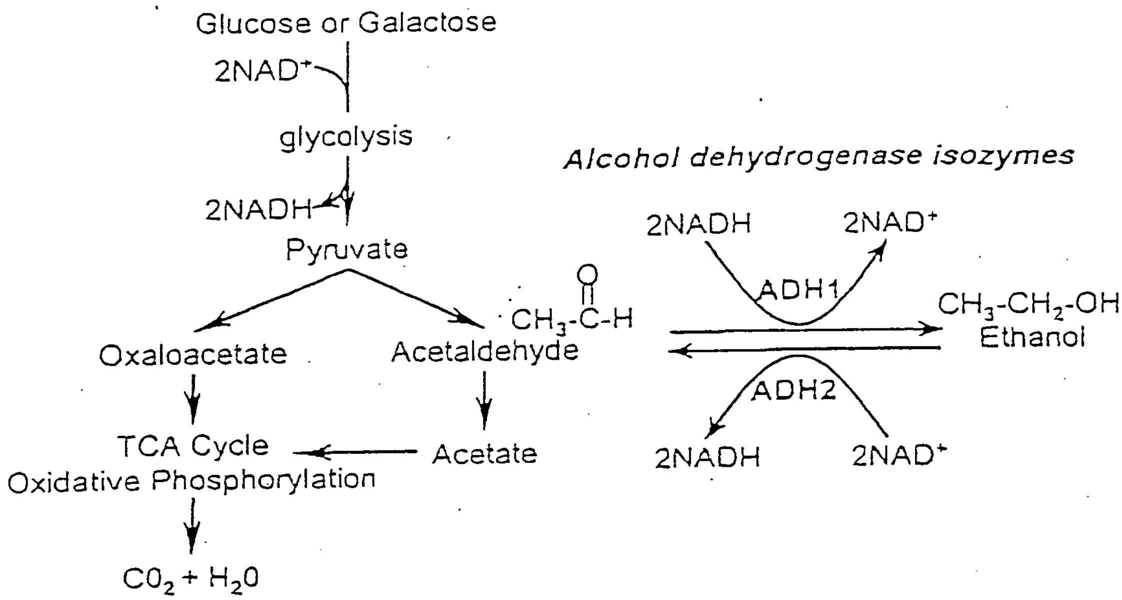


Fig. 4B

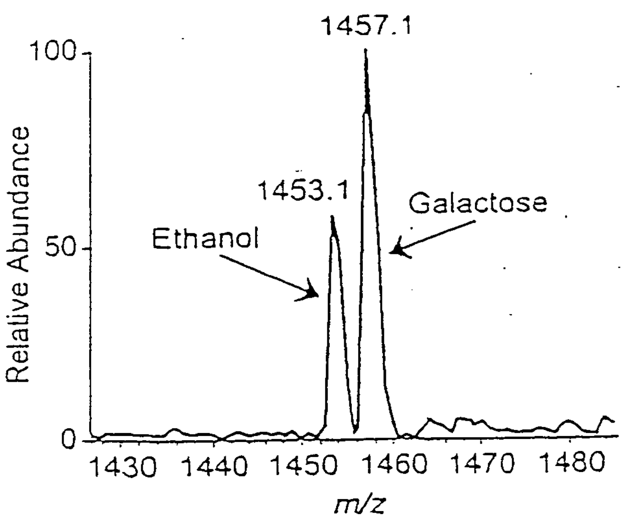
s0319_hlnavcid.0364.0364.2.out
amino acids - 93009033, # proteins - 290043, # matched peptides - 1973750
C:\LCQ\database\owl.v31.3, (C# +494.50)

#	Rank/Sp	(M+H)	C*10^4	Ions	Reference	Peptide
1.	1 / 1	1994.3	4.4675	17/26	G3P_RABIT	(R)VPTPNVSVVDLTC#R (SEQ ID NO:60)
2.	2 / 403	1995.1	2.7366	13/34	SLTRNGL	(E)LGKPVLTANQVTIWEGLR (SEQ ID NO:61)
3.	3 / 3	1995.0	2.6591	16/36	FLP_LACCA	(N)IANPNVYTETLTAATVCTI (SEQ ID NO:62)
4.	4 / 209	1995.0	2.6335	14/36	A42912	(Y)LALLPSDAEGPHGQFVTDK (SEQ ID NO:63)
5.	5 /381	1995.1	2.4634	13/38	H69373	(L)ALLVLVAPAMAAGNGEDLRN (SEQ ID NO:64)

Fig. 5A



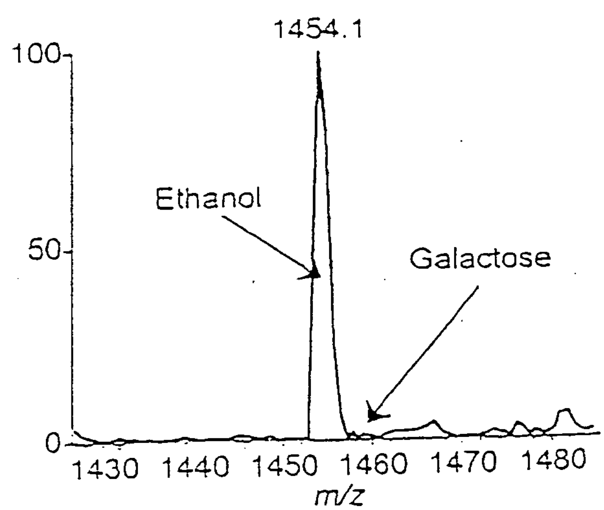
ADH1 : YSVC#HTDLHAWHGDWPLPVK



Ratio: 0.57

Fig. 5B

ADH2 : YSVC#HTDLHAWHGDWPLPTK



Ratio: >200

Fig. 5C

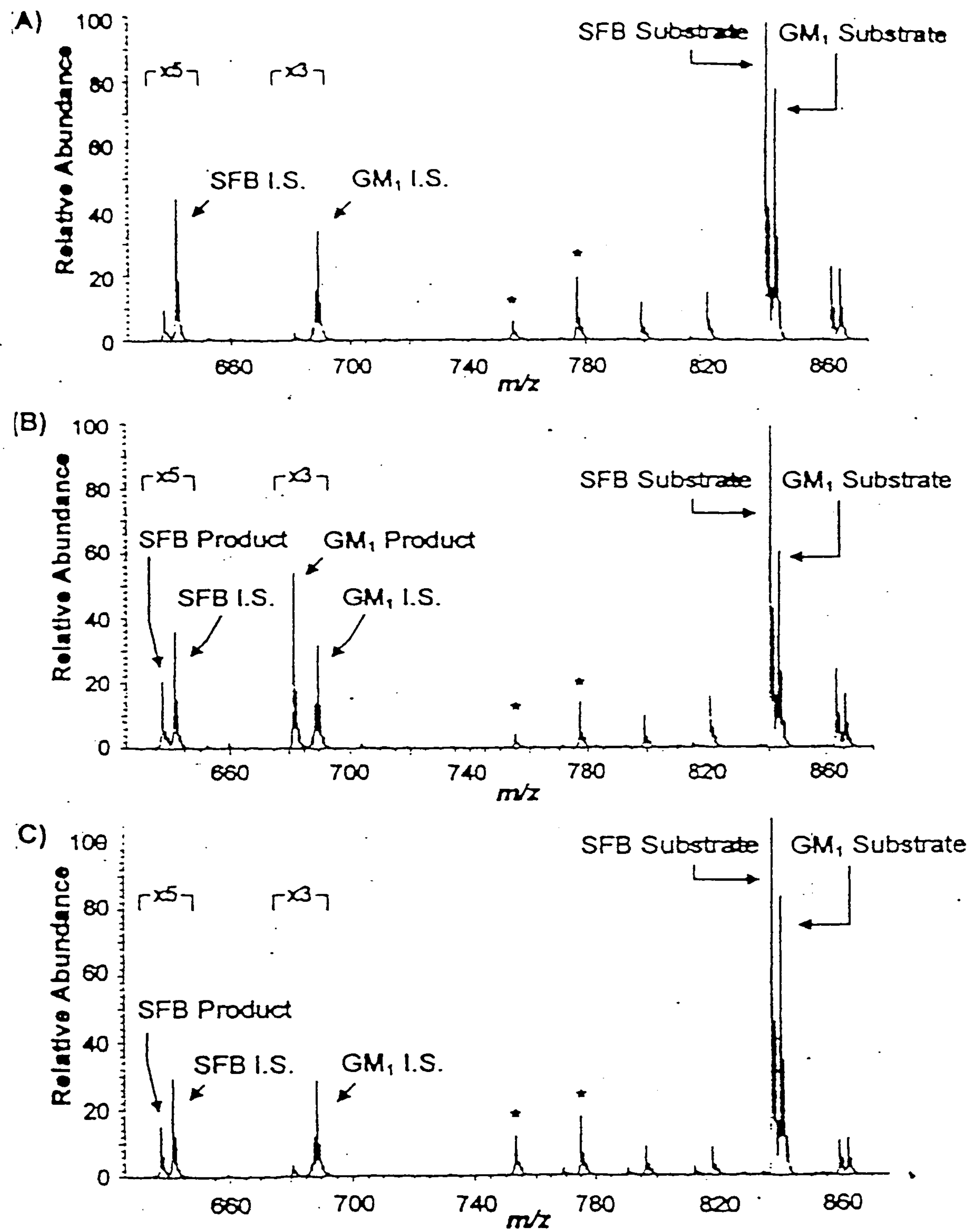

A)
(B)
C)
Relative Abundance
m/z
x5
x3
SFB Substrate
GM$_1$ Substrate
SFB I.S.
GM$_1$ I.S.
SFB Product
GM$_1$ Product
0
20
40
60
80
100
660
700
740
780
820
860

Figure 6A-C

T0024O"h886E860

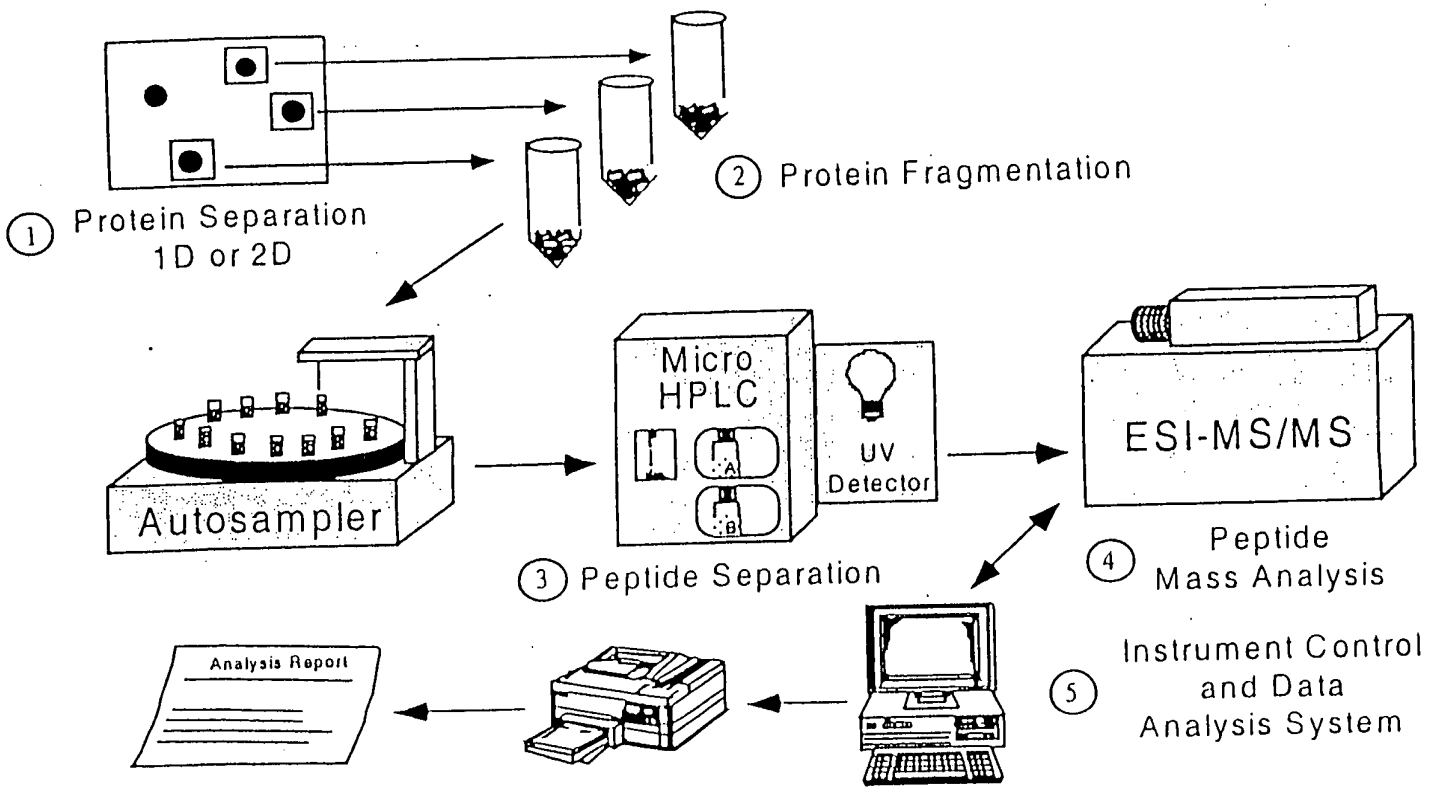


Figure 7 Schematic representation of the automated LC-MS/MS system. Proteins are typically separated by 1D or 2D SDS-PAG (1). Protein spots or bands are selected, excised and proteolytically cleaved with trypsin (2). Digests are loaded into an autosampler, which delivers them sequentially to the injection mechanism of a narrow-bore HPLC system (Michrom). The column gradient is automatically applied to separate individual peptides(3). Column eluate is sprayed directly into a mass spectrometer where sequence information from the peptides is collected (4). Recorded peptide masses and CID spectra are transferred to a data station for Sequest analysis, and a final summary of all identifications made for all samples originally loaded is sent to a printer (5).

T002h0" h886E860

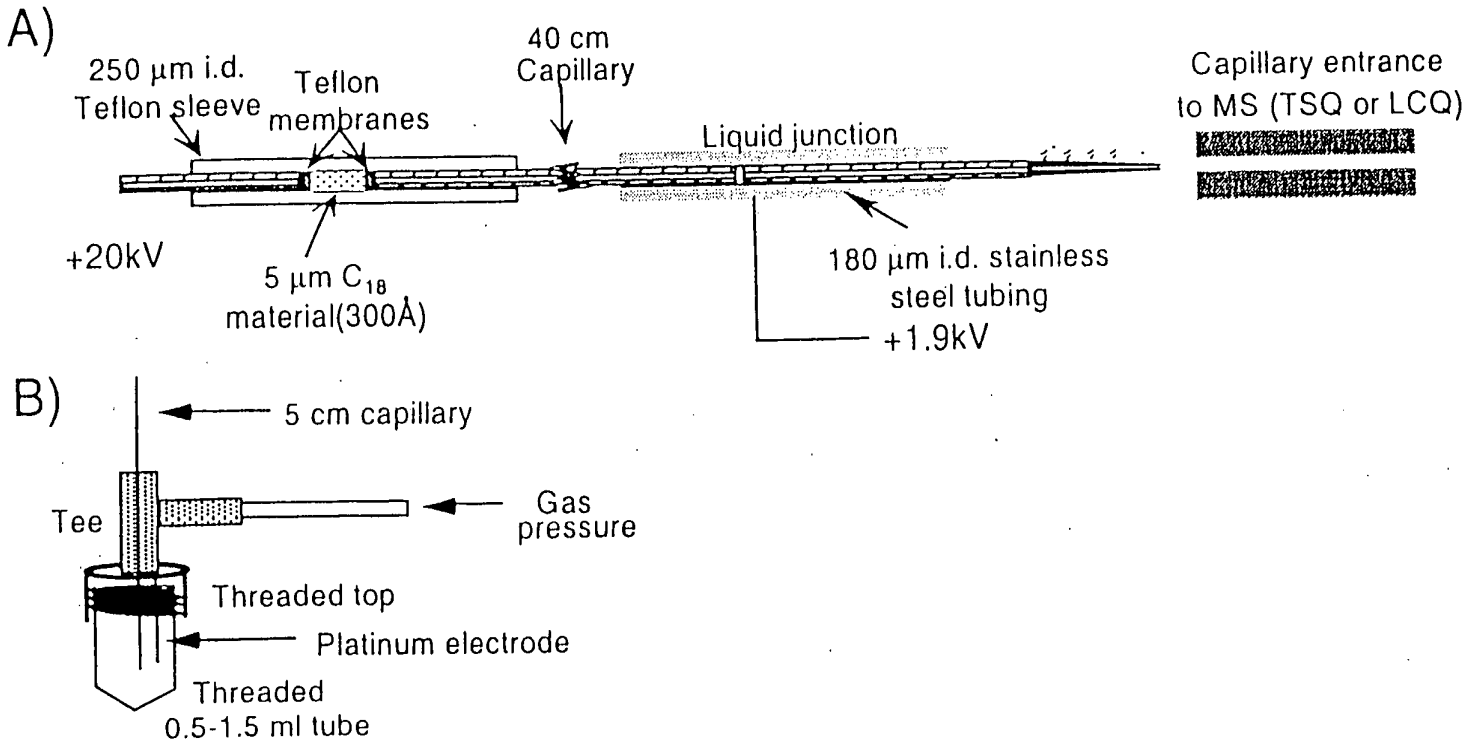


Figure 8 Schematic of the SPE-CE-MS/MS system. A) A fused silica capillary, typically of 50 µm i.d., is modified at the electrospray end with a liquid junction to establish electrical contact with the analytes inside the capillary. Approx. 5 cm from the end of the capillary, the SPE device is introduced. This consists of C_{18}-derivatized, large pore silica beads packed inside a 250 µm i.d. Teflon tubing with Teflon membranes at each end to hold the beads in place between the two fused silica capillaries. B) The injection end of the capillary is inserted into a sealed container which is maintained at a constant, slightly hyperbaric pressure in order to ensure constant flow. A platinum electrode is inserted through the cap, into the container, in order to allow the electrical contact to be made.